BUREAU OF SUGAR EXPERIMENT STATIONS

BRISBANE

QUEENSLAND, AUSTRALIA

TECHNICAL COMMUNICATIONS.

YEAR 1961, No. 3, 4 and 5

No. 3. Bacterial Mottle Disease of Sugar Cane

by

D. R. L. STEINDL

No. 4. Studies with Chlorotic Streak Disease of Sugar Cane
II Transmission in Nutrient Gravel Cultures

by

B. T. EGAN

No. 5. Studies with Chlorotic Streak Disease of Sugar Cane
III Transmission by Short Exposure and Incubation Period of the Disease

by

O. W. STURGESS

Issued by Direction of the Sugar
Experiment Stations Board

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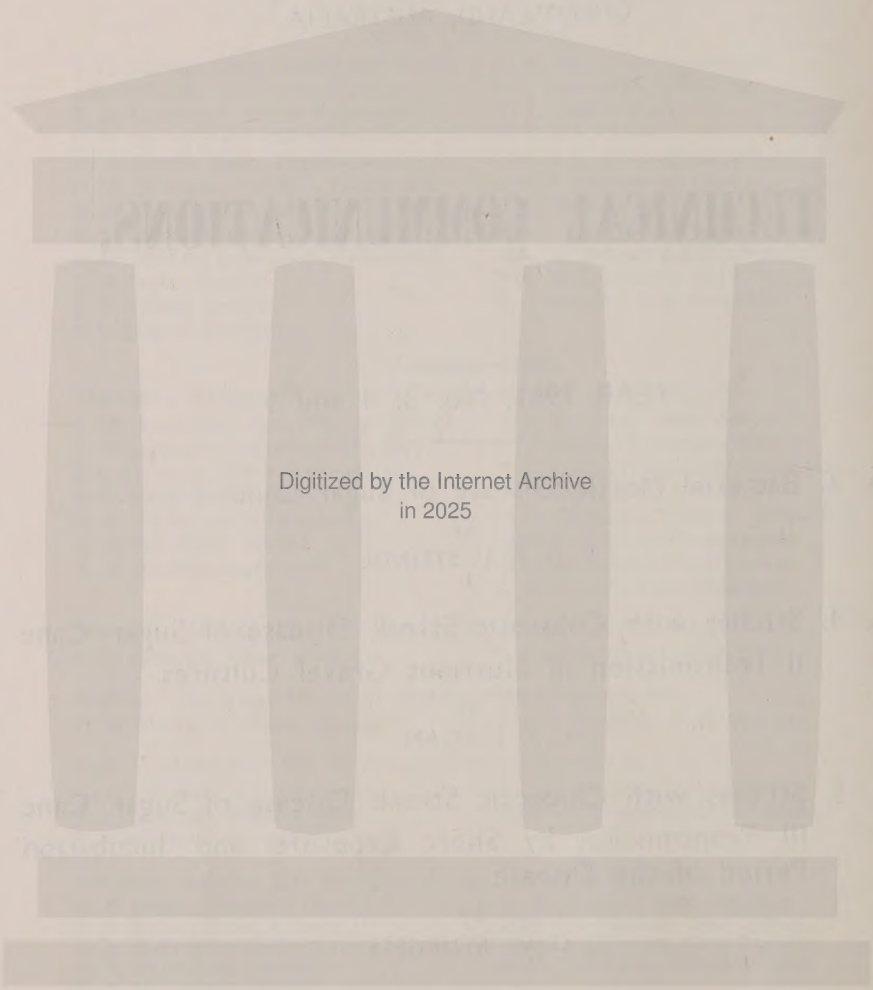

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Bacterial Mottle Disease of Sugar Cane

Caused by *Pectobacterium carotovorum* var. *graminarum* Dowson and Hayward

By D. R. L. STEINDL

SUMMARY

A new bacterial disease of sugar cane causing leaf striping and a chlorotic mottle, together with considerable reduction in growth is described.

The disease has been transmitted experimentally to cane by sett inoculation and by hypodermic needle inoculation into the young spindle. It has been recorded in several grasses and inoculations from cane to grasses, and from grasses to cane, have been successful.

The causal organism has been described and the name *Pectobacterium carotovorum* var. *graminarum* Dowson and Hayward has been proposed for it.

The economic importance of the disease and measures for its control are discussed.

HISTORY AND DISTRIBUTION

Bacterial mottle was first described in Queensland by Steindl [4], although its occurrence had been reported during the previous decade by officers of the Colonial Sugar Refining Company in the Herbert River district of North Queensland. The name "root disease" had been given to it by the Company pathologists because of the poorly developed root system of diseased plants. However, there had been no publication on the disease, and no specific causal organism had been associated with it.

Between 1955 and 1957 the disease was found by Bureau officers in the Giru and Mackay districts, and subsequently its presence has been recorded in all districts from Mossman at the Northern end of the Queensland sugar belt to Plane Creek in the central districts. There are no known records of a similar disease in cane in any country except Queensland; however, a bacterial stalk rot of maize in Egypt is caused by a bacterium which is closely related to the bacterial mottle organism [3].

The occurrence of the disease in several areas suggested that it could become of economic importance, and was the reason for the initiation of investigations into its cause and method of transmission.

SYMPTOMS

Early symptoms of the disease usually consist of creamy-white, regular stripes, one to two millimeters wide, extending from the base, or near the base of the leaf blade, upwards parallel with the vascular bundles. One to many such stripes may occur on a leaf; sometimes they extend the full length of the leaf, but more often cease at irregular intervals along it. As the leaf ages the stripes may enlarge, and orange to rusty-red areas develop within them, sometimes involving the greater portion of the stripes. At this stage the disease has a marked resemblance to the advanced stages of downy mildew, *Sclerospora sacchari* Miy.. When the infection becomes systemic the leaves develop a chlorotic mottling, frequently without any distinct striping. Some varieties show more chlorosis than others, and on occasions almost completely chlorotic shoots are seen. As the leaves age, numerous small, rusty-red flecks and short, narrow stripes appear and, if the leaves are very chlorotic, from a distance they appear quite pink.

Growth of the infected shoots is severely checked, the tips and edges of the leaves wither prematurely, the leaves curl inwards and eventually the whole shoot may die. Diseased stalks frequently produce numerous side shoots at the base, thus giving the effect of excessive tillering. Standover crops have been observed to produce a "witches' broom" effect several feet above ground level when infection has taken place in a well advanced crop. In all cases these adventitious shoots show the characteristic leaf symptoms.

During warm, humid weather numerous, small, whitish globules of bacterial exudate may appear on the under sides of the leaf blades, and the drops of water which accumulate in the spindle and at the dewlap become quite milky because of the presence of immense numbers of bacteria.

Affected plants frequently develop stem galls and multiple buds, but since these conditions also occur with sclerophthora disease, *Sclerophthora macrospora* (Sacc.) Thir. *et al.*, and in otherwise normal cane, they cannot be considered a definite diagnostic characteristic.

Internally, diseased stalks frequently show small, brown, necrotic areas, particularly in the vicinity of the nodes and the growing point. On occasions these areas will break down to form small cavities which frequently contain droplets of a gummy substance containing large numbers of bacteria.

The disease may affect whole stools or any portion of a stool. Sometimes the affected portion will die out thus leaving the remainder of the stool apparently

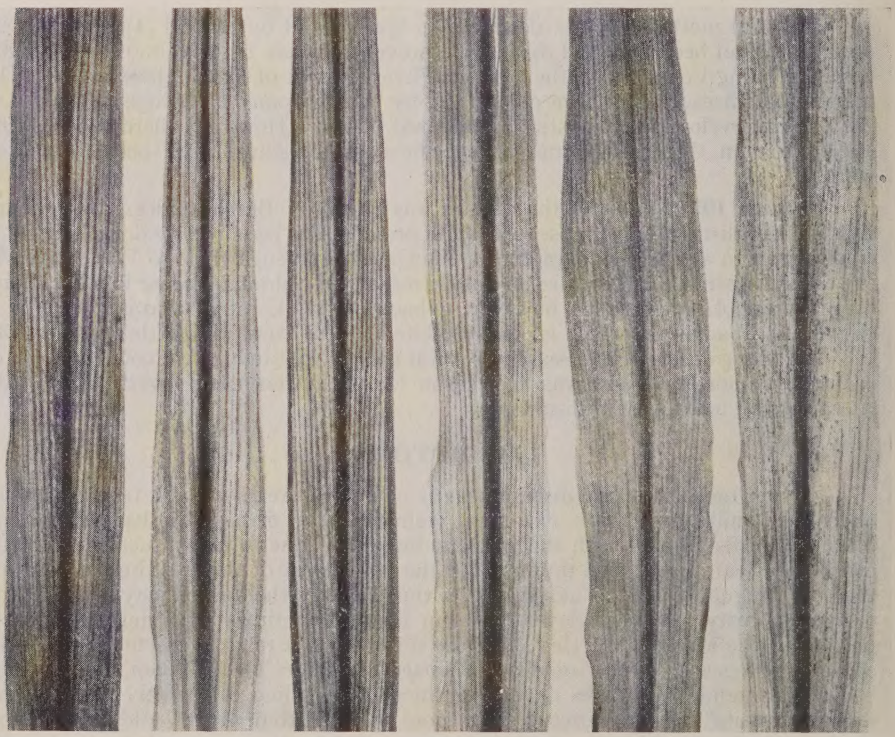

FIGURE 1—Chlorotic mottling and striping caused by systemic infection with bacterial mottle disease in the variety Trojan.

healthy. When a diseased stool is transplanted, and when diseased setts are planted they either fail to germinate or produce only small sickly shoots, which usually die out after a few weeks' growth.

TRANSMISSION

Microscopic examinations of diseased leaves and stems reveal immense numbers of bacteria within the tissues, and a bacterium has been consistently isolated from both leaves and stems of diseased specimens. The pathogenicity of this organism has been shown by a number of inoculations into setts and young shoots of the varieties Q.57 and Trojan.

In the first experiment, 48 single-eye setts of each variety were inoculated by forcing a suspension of a three-day old culture of the organism into the cut ends, and the setts were then planted in pots in the glasshouse. With Q.57, 46 plants germinated, but after reaching a height of 3 or 4 inches, 17 of these plants turned yellow, wilted and died. Of the remainder, five later developed the typical leaf streaks and mottling characteristic of the disease. In the case of Trojan, all plants germinated, two died at an early stage as with Q.57 and three later developed characteristic symptoms. In both varieties the bacterium was readily re-isolated from the infected plants. Control plants were normal.

FIGURE 2—Stunting and excessive tillering in young ratoon Q.66 infected with bacterial mottle.

In a second experiment, 24 young shoots, about 12 inches high, of the varieties Q.57 and Trojan, were inoculated with a suspension of the bacterium by means of a hypodermic needle into the young spindle just above the growing point. After about ten days all inoculated plants were showing a reddened necrotic area around the points of inoculation on the young, unfolding leaves, with a chlorotic mottling extending from this area. The Q.57 plants were more severely affected than the Trojan plants. About three weeks after inoculation, streaks typical of the disease began to develop on all the plants, and again the bacterium was readily isolated. Later a number of the plants began to produce healthy leaves and appeared to be recovering from the disease. However, side shooting then commenced at the base of the inoculated plants, and these side shoots bore characteristic symptoms of the disease. It appears that the bacteria were carried down the leaf sheaths to the buds, which became infected and produced systemically diseased side shoots. Three months after inoculation the Q.57 plants showed only four diseased primary shoots, yet 22 out of 24 plants showed diseased side shoots. Trojan did not show any

FIGURE 3—Witches broom effect caused by bacterial mottle infection in a standover crop of Eros.

disease in the primary shoots at this stage, but 10 out of 20 plants were side shooting. All control plants were normal.

In a third experiment, 16 plants of each of the two varieties Q.57 and Trojan were inoculated by pricking a suspension of the organism into the spindle just above the youngest exposed dewlap, by means of a fine needle. After about 12 days all plants showed creamy-white stripes up to four inches long extending from the inoculation points, and one plant of Trojan showed a chlorotic mottling on the inoculated leaf. The bacterium was re-isolated from several of these streaks. In this experiment symptoms appeared on the inoculated leaves only, and when these leaves reached maturity and died the plants remained healthy.

These experiments were carried out in a glasshouse during the summer months when temperatures and humidities were high, further experiments carried out during the winter months gave a much lower percentage of transmission. This agrees with observations in the field, where transmission occurs mainly during the hot wet season. Natural spread of the disease is definitely associated with flooding, and all known heavily infected fields have been inundated with flood waters at some time. It is probable that flooded infected cane and grasses disgorge through the stomata immense numbers of bacteria, which could be carried to other plants by means of the water and thus set up new infections.

HOST RANGE

A close examination of other plants growing in the vicinity of diseased cane fields has revealed symptoms, similar to those on cane, in three common species of grasses: viz.— elephant grass, *Pennisetum purpureum* Schum., para grass, *Brachiaria mutica* (Forsk.) Stapf. and guinea grass, *Panicum maximum* Jacq.. Symptoms in these grasses consisted of longitudinal chlorotic stripes and a mottle similar to those occurring in sugar cane, but usually without the reddening associated with the disease in cane. Para grass seldom shows the mottling and symptoms in this species are characterized by well defined longitudinal stripes with straight, clear-cut edges. All species show scorching of leaf edges and tips and wilting of shoots in severe cases.

Isolations from these grasses all yielded a bacterium similar to that isolated from cane, and inoculations of each isolate into young shoots of the variety Q.57 produced typical symptoms of the disease.

Isolates from cane were also inoculated into the following plants:—

Maize hybrids Q790 and Q692, Martin and Wheatland grain sorghums, sweet sudan grass (a variety of sudan grass, *Sorghum sudanense* Stapf.), elephant grass, para grass, guinea grass, tomato, cowpea, potato, velvet bean and French bean. In the case of the gramineae, inoculations were made into the spindle just above the growing point by means of a No. 26 gauge hypodermic needle, whilst the dicotyledonous plants were inoculated into the young stem by the same method.

All the gramineae with the exception of para grass became systemically infected and showed the characteristic striping and mottling. The sorghums and sweet sudan grass also showed considerable reddening of tissues similar to cane, and some wilting and death resulted from the infection. Maize also showed some death, preceded by a purplish discolouration of the leaf sheaths and a rotting of the growing point of the stem. Although para grass did not become infected in this experiment there is no doubt that it is widely infected in the field as demonstrated by isolations from it and inoculations into cane.

No infection occurred in any of the dicotyledonous plants.

The disease has been found in one or more of the abovementioned three grasses in most of the areas where it has been found in cane. Hughes [2] reported that bacterial mottle in grasses has been observed in situations where it is improbable that cane has ever been grown, and where flood waters have not come into contact upstream with cane. It is therefore probable that bacterial mottle is essentially a disease of naturally growing grasses rather than cane; it only needs the combination of flooding and susceptible cane varieties to cause infection in commercial crops.

FIGURE 4—Globules of bacterial exudate on the under surface of a leaf of Q.66 infected with bacterial mottle. X3.

THE CAUSAL ORGANISM

On Wilbrink's medium (sucrose 2 per cent, peptone 0.5 per cent, K_2HPO_4 0.05 per cent, $MgSO_4$ 0.025 per cent, agar 1.5-2 per cent) colonies of the causal organism were white, opaque, slightly raised, glistening and butyrous, tending to assume a greyish colour on aging. A preliminary examination showed that it was a gram-negative, multiflagellate, rod-shaped bacterium possessing a number of characteristics of the *Erwinia spp.*. However, cultures were forwarded to Dr. W. J. Dowson at Cambridge University, and to the Commonwealth Mycological Institute, Kew, for identification.

The reactions of sugar cane and maize to the organism were confirmed at Cambridge, and it was found to have most of the biochemical characters of *Pectobacterium carotovorum* (Jones) Waldee (*Erwinia carotovora* (Jones) Holland) but as it infected various grasses it was considered to be a new variety for which the name *Pectobacterium carotovorum* var. *graminarum* is proposed (Dowson and Hayward [1]).

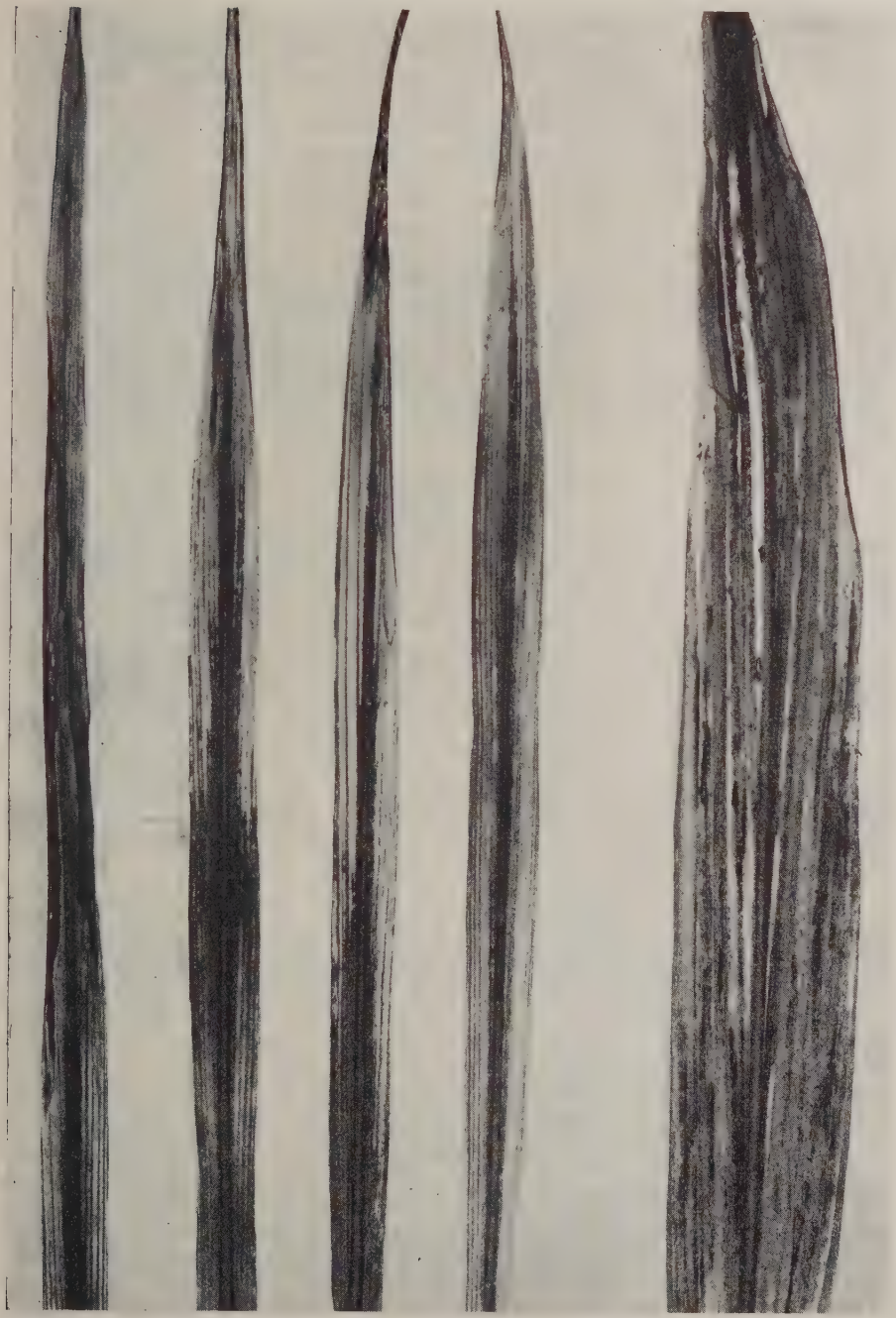

FIGURE 5—Leaves of elephant grass (left) and sorghum (far right) infected with bacterial mottle.

The full description of the organism as supplied by Dowson and Hayward [1] is as follows:—

"Individual cells measure 0.4-0.6μ x 1.5-3.0μ, have 4-6 peritrichous flagella (few cells with more or less flagella) when stained by Bailey's method. Wieringa's pectate gel and gelatin are liquified. Nitrite is formed from nitrate in a synthetic and in a peptone medium containing potassium nitrate. Acid and gas are formed in 24 hours from glucose, sucrose, fructose, galactose, arabinose, mannitol and salicin, from raffinose and lactose after 3-7 days' incubation and acid alone from maltose after about 3 weeks' incubation. The technique used was a modification of that described by Hugh and Leifson [J. Bact., 1953,66,24.]. Aesculin is hydrolysed but not potato starch. No melanin pigment is formed on tyrosine agar. Hydrogen sulphide is produced in a peptone liquid medium supplemented with cysteine hydrochloride. Indole is produced. Citrate and malonate are utilized in Koser's and Leifson's medium respectively. The V.-P. reaction is positive, M.R. negative. Tests for lipase (Sierra [Antonie van Leeuwenhoek J. Microbiol. and Serol., 1957,23,15]) and urease (Christensen [J. Bact., 1946,52,461) were negative. The optimum temperature for growth is about 30°C but the organism will grow at 40°C.."

ECONOMIC IMPORTANCE

Transmission of the disease in the field is usually not rapid, but it is very destructive in plants which do become infected. The disease occurs mainly in low-lying areas, and its spread appears to be associated with flooding; it frequently develops in ratoon crops which have been flooded as plant cane, and is always more prevalent in years when heavy flooding occurs.

Although it is recommended that diseased fields should not be used for planting material the disease is largely self-eradicating, particularly on high land, because of the failure of diseased setts to germinate and the dying out of infected plants.

The disease has probably become more prevalent in recent years because some of the present-day varieties are much more susceptible than the older varieties. Q.57, Q.66 and Trojan are the most susceptible of the present commercial varieties, and all these are suited to localities where the disease spreads most rapidly. It has been recorded in most commercial canes, which show variable degrees of resistance to the disease, although to date no variety is known to be immune to it.

Control measures should consist of the selection of healthy planting material, the destruction of diseased plants and the use of more resistant varieties in localities where the disease is severe. The destruction of infected grasses along water-courses adjacent to cane lands may also have to be considered as a control measure in certain cases.

REFERENCES

[1] Dowson, W. J. and Hayward, A. E. (1960).—The bacterial mottle pathogen of Queensland sugar cane. Int. Sug. J. **62:** 275.

[2] Hughes, C. G. (1958).—58th Ann.Rept., Bur.Sug.Exp.Stas.Qd. 89-90.

[3] Sabet, K. A. (1954).—A new bacterial disease of maize in Egypt. Empire J. exp. Agric. **22:** 65-67.

[4] Steindl, D. R. L. (1957).—Bacterial Mottle.—A new disease of sugar cane in Queensland. Cane Gr. quart. Bull. Qd. **21:** 6-8.

Studies with Chlorotic Streak Disease of Sugar Cane

II. Transmission in Nutrient Gravel Cultures

By B. T. EGAN

SUMMARY

Chlorotic streak disease was transmitted successfully from diseased to healthy plants growing in nutrient-gravel culture in troughs. A comparatively early ratooning of the plants under test resulted in a high transmission rate within a short period of time, although the cane left uncut eventually achieved a similar high rate. Several varieties were tested for symptom expression, growth, and ratooning power under the prevailing conditions; N.Co.310 proved best with Vidar and Q.67 also useful. The use of the systemic insecticide metasystox in the nutrient solution did not affect transmission.

INTRODUCTION

In view of the fact that Abbott and Ingram [1] had reported transmission of chlorotic streak disease by the leafhopper *Draeculacephala portola* Ball, the initial attempts at transmission at the Northern Sugar Experiment Station, Meringa, where the disease is endemic, were set out in an insect-proof house. The growth obtained, however, was abnormal and a large proportion of diseased plants lost their symptoms. In addition, the one-gallon solution containers originally used occupied more space than was readily available. The number of plants handled was multiplied by the use of a series of galvanized iron troughs holding 30 or more plants, while growth was improved by the substitution of gravel culture for straight solutions. The use of troughs also overcame the problem of loss of symptoms since at least a few diseased plants in each trough continued to produce streaks throughout the experiment. It was subsequently felt that, providing adequate controls were maintained, at least preliminary experiments could be carried out in an unscreened house or shed.

MATERIALS AND METHODS

Galvanized iron containers, 1.65 m by 37.5 cm by 22.5 cm deep, were used for all experiments, and were painted inside and out with a bitumastic-based, aluminium paint. An outlet for drainage was provided in the base at one end, and to this a piece of plastic hose was attached. Water levels in the troughs could be checked in a short length of glass tube incorporated in the hose (Figure 1). The troughs were set on bricks placed in shallow trays containing a strong phenyle solution to prevent any soil insect infestation, while the floor, walls and roof of the shed were sprayed with a strong dieldrin solution. A coarse gravel from the bed of the Mulgrave River was used for experimental series I and II, while graded gravel crushings were used for series III. In each case, the gravel was well washed with water before steam sterilization.

The troughs were filled to capacity with gravel, then covered with black polythene sheeting to eliminate algal growth. Three rows of slits were made in the polythene, and through these the shoots were planted at the rate of 32 or 35 per trough. The freshly prepared troughs each held 60 to 65 litres of solution but this amount was progressively reduced as roots and stools developed until, for example, a trough containing a half-grown, first ratoon crop held only 25 to 35 litres.

Apparently healthy cane buds were obtained from varieties growing on the Experiment Station, and were then hot-water treated for 20 minutes at 52°C., before planting in flats of steam sterilized sand in the glasshouse. Varieties used were Badila, N.Co.310, Q.66, Q.67, Trojan and Vidar, all of which are susceptible or highly susceptible to chlorotic streak. Stalks of Q.67 showing many prominent leaf streaks were selected from fields in the Mulgrave and Hambledon mill areas, as a source of diseased plants. Setts from these stalks were washed, soaked in Aretan solution for a few minutes, then planted in flats of sterilized sand. The germinating

FIGURE 1—Young cane in gravel culture in a typical experimental trough.

healthy and diseased setts were kept well separated at all times. After the development of shoot roots, the shoots were excised from the setts and planted in their respective positions. Only diseased shoots showing several leaf streaks were planted out, but these often failed to produce new streaks for some weeks or even months after transplanting. However, no individual trough was without diseased shoots at any stage since some shoots continued to produce streaks throughout the life of the experiments.

All material, whether infected with chlorotic streak or not, was free from ratoon stunting disease.

The complete nutrient solution of Martin [3] was used in Series I and part of Series II experiments, while one similar to that of Sturgess [4] was used in the remainder of Series II and in Series III. Good growth was obtained with both formulae but it was decided to standardize on the latter. The water used was from a reticulated supply originating in a creek flowing through undisturbed rain forest; it is of very high quality and requires no chemical treatment.

TABLE I.

Chlorotic Streak Transmission in Series II.

Variety		Diseased stools at 21st December, 1959				Diseased stools at 7th March, 1960				Grand Total
		Troughs			Total	Troughs			Total	
		A	C	D		A	C	D		
Badila	Plant	0/6	1/6	0/6	1/18					
	R1*	0/2	3/3	1/2	4/7	1/2	3/3	1/2	5/7	13/25
	R2*					3/6	2/5	2/6	7/17†	(52%)
N.Co.310	Plant		0/4		0/4					
	R1	3/3			3/3	3/3			3/3	5/7
	R2						2/4		2/4	(71%)
Q.66	Plant	0/3	0/2		0/5					
	R1		0/2		0/2		0/2		0/2	2/6
	R2					2/2	0/2		2/4†	(33%)
Q.67	Plant			2/7	2/7					
	R1			2/3	2/3			3/3	3/3	10/10
	R2							5/5	5/5†	(100%)
Vidar	Plant	3/6	1/5	0/2	4/13					
	R1	1/2	2/4	2/2	5/8	1/1	3/4	2/2	6/7	13/16
	R2					0/2	1/1	2/2	3/5†	(81%)
Totals	Plant	3/15	2/17	2/15	7/47(15%)					
	R1	4/7	5/9	5/7	14/23(61%)	5/6	6/9	6/7	17/22(77%)	
	R2					8/13	7/14	11/15	19/35(62%)	
Grand Total		7/22 (32%)	7/26 (27%)	7/22 (32%)	21/70 (30%)	13/19 (68%)	13/23 (57%)	17/22 (77%)		43/64 (67%)

*R1 is the early ratooned section, R2 is the ratoon of the plant cane shown, and all diseased stools occurring in plant are included in R2.

†The figures show diseased stools out of total stools. Apparent discrepancies are due to stools failing to ratoon.

The solution was acidified with sulphuric acid to give a pH of 5.0. Initially, ferric citrate was used successfully as the iron source, but a great deal of trouble was experienced at the start of Series III with iron deficiency, and this was not corrected until sodium-iron sequestrol was substituted. Subsequent growth was very good.

Solutions were changed fortnightly until rapid growth of the shoots made weekly changes advisable. Water levels were maintained by the addition of water when necessary, the only aeration being provided by this rise and fall of the solution.

The plants were allowed to stool normally but it was noted that the inside row was usually poorer in this respect than the outer rows. Spray applications of diazinon, dieldrin, DDT and parathion at recommended strengths were used at one time or another to keep insect and mite populations at a minimum.

The initial gravel experiment, consisting of one trough only, was conducted in the insectary. An unscreened shelter shed was erected to house later experiments. This shed had glass sides on the south and east as protection against the prevailing south-easterly winds. At first the roof was of "Windolite" reinforced plastic but it subsequently deteriorated and clear polythene sheeting was substituted.

Experimental Series I.

Planted 11th June, 1958, concluded 8th July, 1959. Seventeen healthy Trojan, eight healthy Badila and seven diseased Q.67 shoots were planted in the one trough in the insectary.

Experimental Series II.

Planted 9th July and 3rd August, 1959, concluded 7th March, 1960. Healthy shoots of Badila, N.Co.310, Q.66, Q.67 and Vidar, plus diseased Q.67 shoots, were planted in three troughs (A, C and D) in the shelter shed, ten diseased and 22 or 25 healthy shoots being planted in each. Additional treatments imposed were—(i) Trough C contained the untreated gravel used in Series I, *i.e.*, it was equivalent to planting in 'diseased' soil; (ii) Trough D was planted 25 days later than A and C. Two troughs (B and E) planted with healthy shoots only were used as controls. Approximately one-third of the shoots in each trough were ratooned on 30th October, *i.e.*, 88 days after planting for Trough D and 113 for the others, and the remainder on 21st December, 1959, *i.e.*, 140 or 165 days after planting.

TABLE II

Chlorotic Streak Transmission in Series III

Diseased stools/total to 19th December, 1960

Variety		Troughs F	Troughs G	Troughs H	Total	Grand Total
N.Co.310	Plant	2/3	4/6	3/3	9/12	
	R1*	3/3	—	3/3	6/6	24/27
	R2*	3/3	3/3	3/3	9/9	(89%)
Q.67	Plant	2/3	2/5	2/3	6/11	
	R1	1/1	—	3/3	4/4	16/21
	R2	3/3	0	3/3	6/6	(76%)
Vidar	Plant	3/3	4/6	3/3	10/12	
	R1	3/3	—	2/3	5/6	23/26
	R2	3/3	2/2	3/3	8/8	(88%)
Totals	Plant	7/9	10/17	8/9	25/35	
	R1	7/7	—	8/9	15/16	
	R2	9/9	5/5	9/9	23/23	
Grand Total		23/25 92%	15/22 68%	25/27 93%		63/74 85%

*R1 is the ratoon of 7th June, R2 that of 15th July.

Experimental Series III.

Planted 5th April, 1960, concluded 19th December, 1960. Twenty-seven healthy shoots of N.Co.310, Q.67 and Vidar, plus eight diseased Q.67 shoots, were planted in each of three troughs (F, G and H) in the shelter shed. Troughs F and G received no further treatment but Trough H received 10 ml. of metasystox (50 per cent w/w active ingredient) every two or three weeks, equivalent to 80 p.p.m. of active ingredient in the solution. This was added at solution changes and allowed to stay in the trough for one week, *i.e.*, until the next solution change. Trough I as control contained 35 healthy shoots of N.Co.310, Q.67 and Vidar. One-third of the shoots in F and H were ratooned on 7th June, 1960, *i.e.*, 63 days after planting, and another one-third on 15th July, *i.e.*, 101 days after planting, when one-third of the shoots in G were also ratooned.

RESULTS

Series I.

Growth was poor and very slow, probably due to the low light intensity experienced in the insectary. It is probable also that the variety Trojan, which is

normally not a very vigorous cane, could not adapt itself to the conditions. Very few secondary shoots were produced and attempts to ratoon shoots ended mostly in failure. However, the first experimental chlorotic streak transmissions ever obtained in Queensland were noted in early November, 1958. Eventually, two of eight Badila stools and one of 15 Trojan stools showed chlorotic streak symptoms.

Series II.

Growth and stooling were quite good in this series (see Figure 2) except for Trough C in which the remains of the previous crop in the unsterilized gravel may have been responsible for the reduced vigour of the plants. In the other troughs the height of primary stalks to the youngest exposed dewlap had reached 0.6 to 1.0 m by mid-December, depending on the variety. Excellent growth was also obtained in the ratoons. Details of the transmissions obtained are shown in Table I, while in Figure 3 the percentage transmission for plant and early ratoon is plotted against time. The average for the three troughs, A, C and D, was used since similar results were obtained in each. The marked superiority of ratoons is evident.

FIGURE 2—Gravel culture gives a satisfactory growth of sugar cane in a shelter shed.

Trough B, which as one of the controls had been planted with healthy shoots only, remained free from disease until 21st December, but towards the end of January chlorotic streak symptoms were noted in a few shoots, and within a month, 80 per cent of the stools showed numerous leaf streaks. Deterioration of the Windolite roofing had occurred and heavy rains in the Christmas period resulted in a considerable water splash between troughs. Trough E which was further removed from splash contamination than B, remained healthy until the conclusion of the experiment. The possible significance of this occurrence of disease in one of the control troughs is discussed below.

Series III.

Growth and stooling were adequate in Troughs F and H but cane in G suffered badly during early growth from iron deficiency and from another, unrecognized deficiency. Resumption of normal growth in Trough G coincided with the cooler weather and the cane in that trough remained at least six weeks behind that in the others. The early ratooning in it was therefore not possible. Lower than normal winter temperatures affected ratooning in both June (R1) and July (R2), mainly in the variety Q.67. Details of the transmissions obtained are set out in Table II, while Figure 3 shows the relation between percentage transmission and time. In

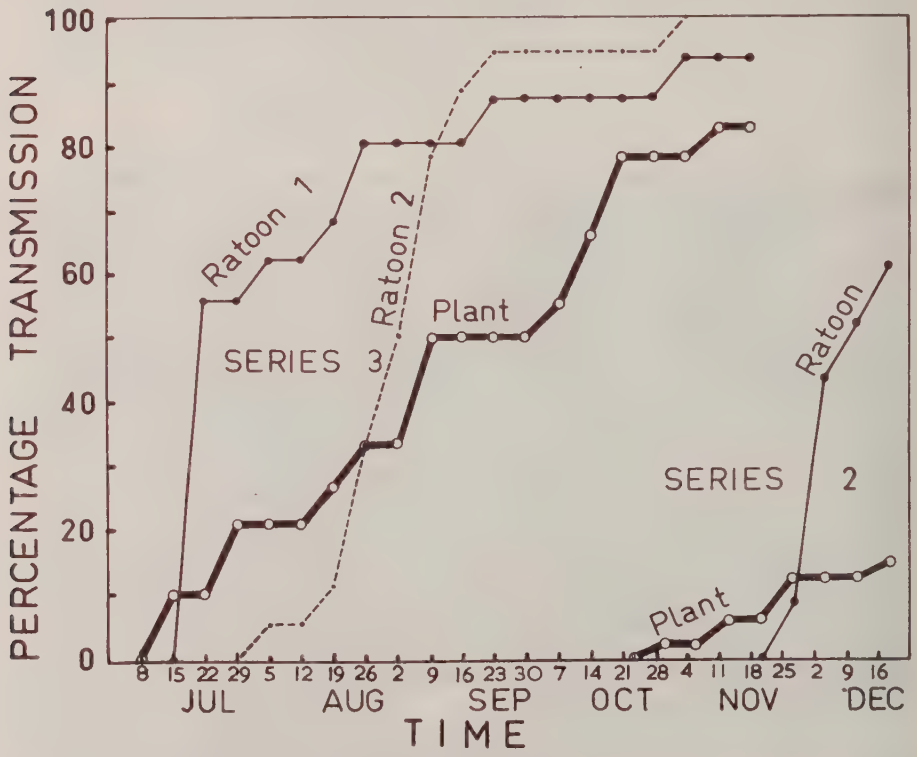

FIGURE 3—The percentage transmissions of chlorotic streak obtained at Meringa in Series 3 and Series 2.

this case the average for troughs F and H only was used since G was not comparable in time because of the initial growth lag, although the results obtained were very similar. The superiority of early ratooning was again demonstrated but the longer period of observation allowed a truer assessment of the potential transmission in plant cane.

The control Trough I remained healthy throughout the life of the experiment.

DISCUSSION

Field observations of chlorotic streak have indicated that the disease is often more severe in the ratoon than in the plant crop. Known healthy cane planted into blocks where the disease is endemic frequently develops few disease symptoms

in the plant crop, and these not until it is almost mature, whereas the young ratoons exhibit numerous streaks. However, where waterlogged soil conditions or flooding occur during the early stages of the plant crop, a heavy chlorotic streak infection may develop within a few months; even so, this usually becomes more severe in ratoons. Length of exposure to the disease does not seem to be the only critical factor involved and some other factor associated with the ratooning process may be concerned.

The results reported here demonstrate that early ratooning does lead to a much higher incidence of the disease in a much shorter time. Thus from Figure 3 it can be seen that the percentage transmission in ratoons rose from 0 to 61 per cent in four weeks while in the same period disease in the plant cane increased from 6 to only 15 per cent. In Series III the advantage of ratoons is still apparent—62 per cent compared with 21 per cent at four weeks or 81 per cent compared with 33 per cent at seven weeks after the first transmissions were noted.

The results suggest that the ratooning factor is of very great importance in the spread of the disease. However, the steady increase in transmission obtained in plant cane should be noted. It seems likely that ratooning does play a part in increasing the rate of transmission but that its major effect could be in accelerating the onset of chlorotic streak symptoms in stools already infected, hence the initial very steep slope in the transmission-time curve for ratoons. The ratooning process has the advantage of forcing the growth of a large number of buds which had been immersed in the nutrient solution and so were presumably capable of being infected by the disease. Also a larger number of shoots per stool become available for inspection, the average for these experiments being six to seven compared with about half that number in plant cane. However, once a shoot becomes infected, most of the other shoots in that stool usually follow suit within a relatively short time.

The length of exposure to infection before transmission can occur and the age of cane which can be ratooned successfully are factors of importance in developing a rapid test for the disease. In order to obtain some information on these points, cane was ratooned at various times after setting up the experiments—88 and 113 days in Series II, 63 and 101 days in Series III. In each case a high transmission rate was obtained, so cane could be ratooned much earlier than 63 days if transmission rate were taken as the criterion of success. Series III experiments were planted in autumn, and growth was slow by the time ratoonings were carried out in winter; the 63 day period was close to the minimum under these conditions and a few stools failed to ratoon. Under the higher temperatures of summer, it is possible that this period could be reduced to 50 days or even less.

The minimum period between planting and the appearance of symptoms in these experiments was 100 days, while for the ratoons the figure was 21 days. Again, such figures are influenced by growth rate of the cane, and under glasshouse conditions Bird *et al.* [2] obtained the figures of 80 and 21 days respectively. Given good conditions for growth, it should be possible therefore to obtain an adequate rate of transmission in ratoons some three months after planting the experiment.

The results obtained by Bird *et al.* [2] in Puerto Rico indicate the early ratooning of the test plants could be beneficial although no plant cane was allowed to remain for comparison. Early ratooning has also been used in further experimental work in progress in Queensland, both in solutions and in soil, and is proving to be of considerable value.

The occurrence of the disease in one of the troughs (Trough B) in Series II (see p. 55) planted to healthy shoots only was of considerable interest.[1] It was apparently not due to primary infection, since the same material had not given a single instance of such infection in the companion control Trough E. The cane in

Trough B had received the same general treatment as the other plantings at all times, but over the Christmas period Trough D (which had already shown a high percentage of transmission) became filled to overflowing with water from a leaking roof and a good deal of this was splashed into Trough B. Trough E was found to be just beyond the reach of splash from D. If the water splash were the actual agent of transmission, infection must have occurred within a very short period since the first leaf symptoms appeared only four to five weeks after the flooding.

Varieties for use in transmission experiments of the type described here must grow and ratoon well in gravel culture, and show a high incidence of chlorotic streak. A range of six varieties—Badila, N.Co.310, Q.66, Q.67, Trojan and Vidar—has been tested to date. Of these, Badila and Q.66 were discarded because of poor growth or insufficiently high disease incidence, while Trojan, which was used only in the initial experiment, gave very poor growth. Q.67 is a variety which is highly susceptible, but in Series III it grew slowly and ratooned poorly under winter conditions. Vidar grew very well in the plant crop but tended to be weak in ratoons, while N.Co.310 was noted for its strong stooling and fast ratooning. On the basis of these observations and the transmission results, N.Co.310 would seem to be the most suitable variety, while Vidar and Q.67 should also be satisfactory. Other varieties have been included in further experimental work in progress.

The re-use of gravel in troughs does not seem to have had any advantage in promoting chlorotic streak transmission where healthy and diseased plants are grown together. On the contrary, reference to Table I shows that percentage transmission was lowest in the Trough C so treated, while growth was much poorer. There is some evidence to indicate that there had been a build-up of adverse factors in the trough, resulting in poor growth of roots.

The addition of metasystox to the trough solution did not affect the chlorotic streak transmission rate, but it did prevent the colonization of any sucking insects or mites. However, it was apparent that the concentration used was too high and some slight phytotoxicity occurred. This method of insect control is simple and efficient, and subsequently has been used in other experiments at half the present reported rate without any apparent ill-effects. The pink mealy bug *Saccharicoccus sacchari* Ckll., cane aphid *Longiunguis sacchari* Zhnt., and the leaf mite, *Tetranychus telarius* L. managed to establish small colonies in troughs not treated with metasystox, while the mid-rib miner *Cosmopteryx dulcivora* Meyr. also was present. Other insects noted as transients included the leafhoppers *Perkinsiella saccharicida* Kirk., *Cicadella albida* Walk., *C. parthaon* Kirk., a small unidentified Jassid commonly found on grass, and the linear bug *Phaenacantha australica* Kirk., although they were noted much less frequently on metasystox treated cane.

These experiments were conducted virtually in the open and so insects had free access to them. Insect transmission of the disease was therefore quite possible but the indications are that it did not occur.

The original aim of these experiments was to develop a reliable and efficient method of transmitting chlorotic streak disease, based on nutrient solutions. The procedure eventually adopted has given quite good results with stool transmissions in the troughs varying from 57 per cent to 93 per cent, with an average of 77. However, by applying the results of these experiments, it should be possible to achieve regularly stool transmissions of 90 per cent if desired, while a test of possible transmission could be completed within 20 weeks.

ACKNOWLEDGMENT

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REFERENCES

[1] Abbott, E. V. and Ingram, J. W. (1942).—Transmission of chlorotic streak of sugar cane by the leafhopper *Draeculacephala portola*. Phytopathology **32**: 99-100.

[2] Bird, J., Cibes, H., and Tio, M. A. (1958).—Transmission of the causal organism of the chlorotic streak disease of sugar cane through the roots of plants grown in nutrient solution. Uni. Puerto Rico Agric.Exp.Sta. Tech. Paper No. 27.

[3] Martin, J. P. (1934).—Symptoms of malnutrition manifested by the sugar cane plant when grown in culture solutions from which certain essential elements are omitted. Hawaii. Plant. Rec. **38**: 3-31.

[4] Sturgess, O. W. (1960).—Studies with chlorotic streak disease of sugar cane. Bur.Sug. Exp.Stas. Qd. Tech. Comm. 1960. No. 3.

Studies with Chlorotic Streak Disease of Sugar Cane

III. Transmission by short exposure and incubation period of the disease

By O. W. STURGESS

SUMMARY

Chlorotic streak transmission occurred during periods of association of seven to twelve days of diseased and healthy sugar-cane plants growing together in either aerated nutrient or gravel-nutrient solutions. The incubation period under the conditions of the experiments ranged from 72 to 308 days with a mean period of 166 days.

INTRODUCTION

The success of previous transmission studies with chlorotic streak [1, 2] made possible the determination of the incubation period of the disease under controlled conditions. This was carried out in two experiments in a glasshouse in Brisbane extending from the summer of 1960 until the autumn of 1961.

MATERIALS AND METHODS

Healthy test plants of the varieties H.32-8560, Ebene 1/37, and N.Co.310 were raised by germinating healthy setts, previously hot-water treated at 52°C for 20 minutes, in steam-sterilized sand. Rooted shoots severed from these setts were transferred to aerated solutions and the more advanced shoots later selected. Chlorotic streak plants, expressing systemic foliar symptoms, which had been raised in a similar manner to the above, were used as a disease source. Nutrient solutions were prepared and maintained in accordance with the procedure described in a previous communication [2].

Experiment A:—

On February 24, 1960, ten chlorotic streak diseased plants were established in separate, aerated solution containers of one-gallon capacity. A healthy test plant was added to each as a companion plant for a period of seven days. Test plants were then transferred to individual, gravel-nutrient cultures (*see* Figure I). During successive weeks, a total of five groups of ten plants each was used.

Experiment B:—

Ten diseased plants were established in a large, gravel-nutrient trough (*see* Figure 2) and, commencing on April 27, 1960, three successive groups of ten test plants were added to the trough for periods ranging from eight to twelve days. After the exposure to infection, test plants were transferred, as in Experiment A, to separate, gravel cultures.

During the experiments each test plant was ratooned at least twice, for two reasons:—firstly, to encourage an early expression of symptoms by ratoon shoots and, secondly, to limit plant heights in the glasshouse during the summer months. The experiments were terminated on April 24, 1961.

RESULTS

The number of transmissions obtained and the period for symptom development for each infected test plant are summarized in Table I. In Experiment A, only one of the five groups developed chlorotic streak while in Experiment B all three groups

FIGURE 1—Gravel-nutrient container of one-gallon capacity in which treated test plants were grown.

FIGURE 2—The gravel-nutrient trough used in Experiment B: chlorotic streak plants on the left and test plants on the right.

did so. A total of 17 plants became infected in the two experiments. After making allowance for the days of association in the common cultures, the incubation period is shown to range from a minimum of 72-79 days to a maximum of 300-308, with a mean period of 166 days.

TABLE I

Chlorotic streak transmission rates and incubation periods for foliar symptom development.

Experiment	Variety (No. plants)	Exposure to disease, days	Test plants lost	Transmission	Incubation period, days
A1	H.32–8560 (10)	7	2	0	—
A2	H.32–8560 (10)	7	0	0	—
A3	H.32–8560 (6) Ebene 1/37 (4)	7	1	0	—
A4	Ebene 1/37 (10)	7	0	0	—
A5	Ebene 1/37 (10)	7	0	1 2 1 1	72– 79 128–135 173–180 260–267
B1	N.Co.310 (10)	12	1	1 2 1 1	98–110 112–124 120–132 248–260
B2	N.Co.310 (10)	8	0	2 1	280–288 300–308
B3	N.Co.310 (10)	8	0	1 2 1	82– 90 104–112 153–161
				17 (total)	166 (mean)

DISCUSSION

In Puerto Rico [1] and Queensland [2], chlorotic streak disease has been transmitted by growing diseased and healthy plants continuously in common nutrient solutions. The experiments detailed in the present paper were designed to ascertain whether disease transmission occurred during comparatively short periods of association of diseased and healthy root systems and, if it did occur, records were to be kept on the elapse of time before the appearance of symptoms.

The results obtained, as shown in Table I, demonstrate that short periods of association ranging from seven to twelve days, are sufficient for the transmission of chlorotic streak disease. It is obvious that little significance can be attached to the differences in the numbers of transmissions obtained between different groups since so many variables were involved. These included the time of the year, the variety, the length of association and whether the association was with single plants or a group of plants. It should be noted, however, that the first four groups in Experiment A were all negative and that the last in that experiment and all three in Experiment B were positive.

In Experiment A, the different transmission rates recorded for the plants of Ebene 1/37 in treatments A4 and A5 are worthy of some comment. The treatments differed only in the time interval of one week elapsing between the commencement of each exposure to infection. The negative results of A4 (0/10 shoots) when compared with the positive in A5 (5/10) imply that the infection mechanism is delicately balanced and may be affected by such slight environmental factors as minor variations in the temperatures of the nutrient solutions or the atmosphere, or in the light falling on the plants. It is possible that the environmental factors may have an effect on the release of inoculum from diseased roots, the infectivity of the inoculum or the capacity of healthy roots to withstand infection, and further investigation is warranted.

The wide variation in incubation period from plant to plant obtained in the experiments does nothing to add to an understanding of the mechanism underlying the reaction of the plant to chlorotic streak disease and such a variation could indicate that an even wider range is possible under other conditions.

Limitations of space in the glasshouse for these long-term experiments prevented the establishment of a control series in which the conditions of the experiments were exactly reproduced; however, a gravel-nutrient trough containing healthy plants of the variety N.Co.310 did not develop disease when maintained for the full period in the house in close proximity to the test containers, nor did a series of similar controls for other experiments under way at the same time.

REFERENCES

[1] Bird, J., Cibes, H. and Tio, M. (1958).—Transmission of the causal agent of the chlorotic streak disease of sugar cane through the roots of plants grown in nutrient solution. Univ. Puerto Rico Agric. Expt. Sta. Tech. Paper No. 27.

[2] Sturgess, O. W. (1960).—Studies with chlorotic streak disease of sugar cane. 1 Introductory nutrient solution studies. Bur.Sug.Expt.Stas. Qd. Tech. Comm. 1960. No. 3.

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